

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/205</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/06795</b> <b>(43) International Publication Date:</b> 27 February 1997 (27.02.97)
<b>(21) International Application Number:</b> PCT/LV96/00003 <b>(22) International Filing Date:</b> 20 August 1996 (20.08.96) <b>(30) Priority Data:</b> P-95-255 21 August 1995 (21.08.95) LV <b>(71)(72) Applicants and Inventors:</b> KALVINSH, Ivars [LV/LV]; Apartment 8, Miera 17, LV-2169 Salaspils (LV). VEVERIS, Maris [LV/LV]; Apartment 20, Vejavas 10/2, LV-1035 Riga (LV). <b>(74) Agent:</b> FOGEL, Abraham; Alfa-Patents, Marstalu 2/4, LV-1050 Riga (LV).		<b>(81) Designated States:</b> CA, JP, UA, US, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>
<b>(54) Title:</b> PHARMACEUTICAL COMPOSITIONS CONTAINING GAMMA-BUTYROBETAIN FOR TREATMENT OF BLOOD FLOW DISORDERS  <b>(57) Abstract</b> <p>The invention relates to <math>\gamma</math>-butyrobetaine-containing pharmaceutical compositions for oral, parenteral, subcutaneous or rectal administrations, that are providing for the treatment blood circulation disturbances of various genesis and localisation. This composition in the experiments on anaesthetized cats at a dose of 10 mg/kg, i.v. increases the total blood flow by 12 %, not considerably changing blood pressure and heart rhythm. The composition arrests adrenaline-induced isolated rabbit ear blood-vessel spasms. In a concentration of 2.0 mM it decreases reperfusion pressure by 18 %. NO-synthase blocking reverses the composition effect on adrenaline-caused blood-vessel spasms. Being infused the composition at a dose of 200 mg/kg significantly increases blood coagulation during phases I-II. In the comparative experiments the disclosed composition demonstrates more potent effect compared to known medicine [3-(2,2,2-trimethylhydrazinium)propionate]. The preparation is distinguished by a low toxicity and wide safety margin.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## DESCRIPTION

### PHARMACEUTICAL COMPOSITIONS CONTAINING GAMMA-BUTYROBETAIN FOR TREATMENT OF BLOOD FLOW DISORDERS

#### TECHNICAL FIELD

The present invention relates to pharmaceutical compositions, namely to the pharmaceutical compositions which are providing for treatment of blood flow disturbances of various genesis and localisation. The therapeutic composition contains the known chemical substance, the novel action of which gives unexpected pharmacological effects, namely, there is disclosed pharmaceutical composition which contains  $\gamma$ -butyrobetaine as an active principle in a combination with pharmaceutically acceptable fillers and/or solvents.

#### BACKGROUND ART

$\gamma$ -Butyrobetaine (actinine), from which the mammalian organism synthesises carnitine, was primarily characterised as a toxic substance which accelerates respiration, causes salivation and lacrimation, pupil dilation, vasoconstriction and heart stop in diastole (W.Linneweh, Z.Physiol.Chem., 42,181,1929). At the same time, in later papers other authors ascertained that  $\gamma$ -butyrobetaine is extremely low toxic ( $LD_{50}$  7000 mg/kg, s.c.). (W.Rotzsch, L.Lorenz,E.Strack, Acta biol.med.ger. 1959, 3, 28-36). Literature lacks the data on cardiovascular effects of  $\gamma$ -butyrobetaine, though it was reported (Hosein E.A., McLennan H. Pharmacological action of  $\gamma$ - butyrobetaine. Nature, 1956, 183, 328-329) that butyrobetaine is a substance similar to acetyl choline with a

prolonged action. However, later the same authors reported that by an error the experiments involved, instead of  $\gamma$ -butyrobetaine, its methyl ester which in fact possesses cholinergic properties. Contrary to the former  $\gamma$ -butyrobetaine was characterised as a pharmacologically inert substance (E.A.Hoseln, P.Proulx, Isolation and probable functions of betaine esters in brain metabolism, *Nature*, 1960, 187, 321-322. A.S.V.Burgen, F.Hobiger. *Brit.J.Pharmacol.*, 1949, 4, 229. E.Strack, K.Foesterling. *Z. Physiol. Chem.*, 1953, 295, 377

The closest structural analogue of  $\gamma$ -butyrobetaine which is used for the treatment of cardiovascular diseases is  $\gamma$ -betaine aza-analog - 3-(2,2,2-trimethylhydrazinium)propionate (Mildronate, Quaterine). Its mechanism of action is based on limitation of carnitine biosynthesis rate and related long-chain fatty acid transport decrease through mitochondria membranes [Simkhovich B.Z., Shutenko Z.V., Meirena D.V. et al. 3-(2,2,2-trimethylhydrazinium)propionate (THP) - a novel  $\gamma$ -butyrobetaine inhibitor with cardioprotective properties. *Biochem.Pharmacol.* 1988, 37, 195-202].

#### DISCLOSURE OF THE INVENTION

The cardiovascular activity and the toxicity of pharmaceutical compositions containing  $\gamma$ -butyrobetaine was determined.

Acute toxicity was evaluated on male and female mice (19-26 g), 10 animals in a group. The substances were administered in the form of 10% solution in water or in isotonic solution orally or intravenously (with 0.004 ml/sec rate, if i.v.). It was established that at oral administration  $LD_{50}$  of  $\gamma$ -butyrobetaine is >4500 mg/kg, but at intravenous injection  $LD_{50}$  is 1860(1430-2418) mg/kg, which testifies that  $\gamma$ -butyrobetaine is practically non-toxic substance. Special experiments on cats demonstrated that the pharmaceutical compositions containing purified  $\gamma$ -butyrobetaine at a dose 186 times lower than toxic possesses a stronger effect on blood vessel tonus and blood flow than the known preparation and closest structural analogue Mildronate, and, contrary to acetyl choline, there are not observed blood pressure decrease and heart rate decline, while blood flow essentially increases (Table 1).

**Table 1. Influence of 3-(2,2,2-trimethylhydrazine)propionate (M),  $\gamma$ -butyrobetaine (GBB) and acetylcholine (Ach) on haemodynamics in anaesthetized cats**

Substance	Dose, i.v., mg/kg	Blood pressure changes, %	Pulse rate changes	Blood flow rate changes, %
M	5.0	- 3	- 3	+ 5
M	10.0	- 5	- 3	+ 8*
GBB	5.0	- 4	- 5	+ 6
GBB	10.0	- 7 - +3	- 5	+ 12**
Ach	0.001	-35*	-20*	- 8

\*  $p < 0.05$  vs the initial parameters

\*\* $p < 0.05$  vs the corresponding M dose

The experiments were performed on male and female (2.9-3.8 kg) anaesthetized cats (urethane (200mg/kg) and chloralose (50 mg/kg), both i.p.).

The chest was opened in the experimental animals, they were artificially respirated, and blood pressure in the carotid artery as well as general aorta blood flow were measured on physiograph DMP-4B of "Narco Bio-Systems", USA.

If the observed  $\gamma$ -butyrobetaine effect on the blood flow was connected with earlier erroneously attributed cholinergic component which, mainly relates to  $\gamma$ -butyrobetaine ester (The Merck Index, Eleventh Edition, 1871) impurities in the samples of insufficiently purified  $\gamma$ -butyrobetaine, then one would anticipate a significant decrease in the blood pressure and heart rate (see acetyl choline effect, Table I). The observed cardiovascular effect indicates a positive inotropic effect of the proposed therapeutic composition with simultaneous peripheral resistance decrease

by a completely another mechanism, which can be used in the treatment of low heart potency and of blood circulation disturbances of various genesis.

In the experiments with isolated rabbit ear blood vessels the pharmaceutical composition which contains  $\gamma$ -butyrobetaine was 2-3 times more potent in adrenaline-induced blood vessel spasm than the closest structural analogue - the known preparation 3-(2,2,2-trimethylhydrazinium)propionate (M).

**Table 2. Influence of 3-(2,2,2-trimethylhydrazine)propionate (M) and  $\gamma$ -butyrobetaine (GBB) on the blood vessels spasms induced by adrenaline in the isolated rabbit's ear**

Substance concentra- tion ( $\mu$ M)	Systolic pressure (mm Hg) max/min				Decrease of the systolic pressure, %
	Initial parameters		Parameters after adrenaline injection $3 \cdot 10^{-7}$ M		
	max	min	max	min	
M, 0.3	38 $\pm$ 5	8 $\pm$ 2	125	80	1
M, 1.0	38 $\pm$ 5	8 $\pm$ 2	123	77	4
M, 2.0	38 $\pm$ 5	8 $\pm$ 2	126	80	8*
GBB, 0.3	38 $\pm$ 5	8 $\pm$ 2	124	76	6
GBB, 1.0	38 $\pm$ 5	8 $\pm$ 2	125	80	15*
GBB, 2.0	38 $\pm$ 5	8 $\pm$ 2	125	78	18*

\*  $p < 0.05$

\*\*  $p < 0.01$

It was unexpectedly discovered that in the basis of this vasodilating effect lies NO-synthase activation which is completely blocked by L-NO<sub>2</sub>-arginine (Table 3).

**Table 3. Influence of 3-(2,2,2-trimethylhydrazine)propionate (M) and  $\gamma$ -butyrobetaine (GBB) on the blood vessels spasms induced by adrenaline in the presence of L-nitroarginine (L-NO<sub>2</sub>-Arg) (10 mg/l) in the isolated rabbit's ear**

Substance concentra tion (μM)	Systolic pressure (mm Hg) max/min				Decrease of the systolic pressure, %
	Initial parameters		Parameters after adrenaline injection 3.10 <sup>-7</sup> M		
	max	min	max	min	
M, 0.3	36±5	7±2	165	102	0
M, 1.0	36±5	7±2	163	100	0
M, 2.0	36±5	7±2	165	100	2
GBB, 0.3	36±5	8±2	168	105	0
GBB, 1.0	36±5	8±2	165	100	0
GBB, 2.0	36±5	8±2	163	100	0

\*  $p < 0.05$  vs the initial parameters

$\gamma$ -Butyrobetaine also affects blood coagulation time. This was determined in male ICE-JCL albino mice (24-28 g), 10 mice in a group, using Moravic's method (Thodorov Y. *Khlinicheskoye laboratornoye issledovaniya v pediatrii, Medic.Phys.*", Sophia, 1966, p.p.479-480, in Russian). Time when fibrin strings develop was determined. The blood was sampled from jugular vein, mice were preliminarily anaesthetized with urethane (1000 mg/kg, i.p.). The solutions of the substances were infusively administered directly before detection of the blood coagulation time.

Table 4 shows that  $\gamma$ -butyrobetaine considerably prolongs blood coagulation I-II phase, i.e. the time when fibrin strings develop. This means that pharmaceutical compositions on the butyrobetaine basis can be applied in the therapy of such blood circulation failures which are connected with thrombus formation and thrombus embolia.

**Table 4. Influence of  $\gamma$ -butyrobetaine (GBB) on blood coagulation time in mice (after Moravica)**

Substance, dose (mg/kg)	Coagulation time (sec)
GBB, 200 mg/kg, injection	46 + 5.5*
Control (isotonic solution)	23.75 + 3.4

\* $p < 0.05$

Thus, we have unexpectedly discovered that the pharmaceutical composition on the basis of  $\gamma$ -butyrobetaine possesses a wide spectrum of vascular action which is connected with its effect on blood vessel and miocardium tonus as well on NO-synthase, being more potent than known preparation Mildronate which is a close  $\gamma$ -butyrobetaine structural analogue. Hence, the pharmaceutical composition containing  $\gamma$ -butyrobetaine is a promising agent for the treatment of blood flow disturbances of various genesis. The preparation can be administered both orally, parenterally, rectally or transcutaneously.

In the case the active principle is administered as injection or orally in the form of drops, syrup or drink the pharmaceutical composition contains  $\gamma$ -butyrobetaine in the total amount of 0.5 to 40% by weight, and as a pharmaceutically acceptable solvent - distilled water, isotonic or glucose or buffer solution.

In the case the active principle is administered orally or sublingually in tablets, caplets, dragee, granules, powders or capsules they contain  $\gamma$ -butyrobetaine in total amount of 0.01 to 0.5 g in a tablet, caplet, dragee, capsule or in one portion of powder or granule.

In the case the active principle are administered transcutaneously its content in an ointment or plaster makes up 0.5 to 40% by weight. In the case the active principle is administered rectally its content in a suppository or microenema accounts for 0.5 to 40% by weight.



## CLAIMS

1. A pharmaceutical composition for the treatment of blood flow disturbances, which contains  $\gamma$ -butyrobetaine as an active principle and pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to Claim 1, wherein the composition contains 0.5-95% of  $\gamma$ -butyrobetaine by weight.

3. The pharmaceutical composition according to Claim 1 or 2 wherein it is intended for oral or sublingual administration and is in the form of tablets (with or without a cover), capsules, caplets, dragees, granules, powder or solution, which contain 0.01-0.5g of active principle by weight in every tablet, capsule, dragee, granule or powder dose, or also this is a 0.5-40% solution or syrup for oral administration.

4. The pharmaceutical composition according to Claim 3, wherein the acceptable carrier is selected from the group of substances which consist of stearinic acid and its salts, lactose, glucose, saccharose, starch, talc, vegetable oils, polyethylene glycols, microcrystalline cellulose, aerosil, aromatizers, flavoring agents, colorants, ethyl alcohol and water, which are taken separately or are used in combinations.

5. The pharmaceutical composition according to Claim 1, wherein it is designed for parenteral administration and it has a solution form for injections, which contain 0.5-40% of the active principle by weight and pharmaceutically acceptable solvent.

6. The pharmaceutical composition according to Claim 5, wherein a pharmaceutically acceptable solvent is selected from the group of solvents which contain a distilled water, isotonic solution, buffer solution or glucose solution, which are taken separately or are used in combinations.

7. The pharmaceutical composition according to Claim 1 or 2, wherein it is intended for transcutaneous administration of the active

principle and it is in the form of ointment, solution or plaster, which contains 0.5-40% of the active principle by weight and pharmaceutically acceptable carrier.

8. The pharmaceutical composition according to Claim 7, wherein the pharmaceutically acceptable carrier is chosen from the group which consists of water, polyethylene glycols 400, 1500 and 4000, vegetable oils, fats, glycerine, preservatives, emulgators, stabilisers, porous polymer material, dimethylsulphoxide, alcohol and water which are taken separately or are used in combinations.

9. The pharmaceutical composition according to Claim 1 or 2, wherein it is meant for rectal administration of the active principle in the form of suppositories or microenema, which contain 0.5-40% of the active principle by weight and pharmaceutically acceptable carrier.

10. The pharmaceutical composition according to Claim 9, wherein the pharmaceutically acceptable carrier is selected from the groups which consists of water, polyethylene glycols 400, 1500 and 4000, vegetable oils, fats, glycerine, preservatives, emulgators and stabilisers, which are taken separately or used in combinations.

**AMENDED CLAIMS**

[received by the International Bureau on 16 January 1997 (16.01.97);  
original claim 1 amended; remaining claims unchanged (1 page)]

1. A pharmaceutical composition for the treatment of blood flow disturbances, not induced by L-carnitine deficiency, which contains  $\gamma$ -butyrobetaine as an active principle and pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to Claim 1, wherein the composition contains 0.5-95% of  $\gamma$ -butyrobetaine by weight.

3. The pharmaceutical composition according to Claim 1 or 2 wherein it is intended for oral or sublingual administration and is in the form of tablets (with or without a cover), capsules, caplets, dragees, granules, powder or solution, which contain 0.01-0.5g of active principle by weight in every tablet, capsule, dragee, granule or powder dose, or also this is a 0.5-40% solution or syrup for oral administration.

4. The pharmaceutical composition according to Claim 3, wherein the acceptable carrier is selected from the group of substances which consist of stearinic acid and its salts, lactose, glucose, saccharose, starch, talc, vegetable oils, polyethylene glycols, microcrystalline cellulose, aerosil, aromatizers, flavoring agents, colorants, ethyl alcohol and water, which are taken separately or are used in combinations.

5. The pharmaceutical composition according to Claim 1, wherein it is designed for parenteral administration and it has a solution form for injections, which contain 0.5-40% of the active principle by weight and pharmaceutically acceptable solvent.

6. The pharmaceutical composition according to Claim 5, wherein a pharmaceutically acceptable solvent is selected from the group of solvents which contain a distilled water, isotonic solution, buffer solution or glucose solution, which are taken separately or are used in combinations.

7. The pharmaceutical composition according to Claim 1 or 2, wherein it is intended for transcutaneous administration of the active



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/LV 96/00003

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/205

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,4 382 092 (CLAUDIO CAVAZZA) 3 May 1983	1,3
Y	see column 1, line 7 - line 18 see column 3, line 27 - column 4, line 60 ---	2,4-10
X	US,A,5 030 458 (AUSTIN L. SHUG ET AL.) 9 July 1991	1
Y	see column 2, line 10 - line 17	2-10
Y	see column 5, line 51 - column 6, line 63 ---	2-10
Y	US,A,4 474 812 (CLAUDIO CAVAZZA) 2 October 1984 see column 1, line 5 - line 24 see column 4, line 10 - line 11 ---	1-10
Y	US,A,4 451 485 (IVARS Y. KALVISH ET AL.) 29 May 1984 see column 2, line 4 - line 9 ---	1-10
-/-		



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

29 October 1996

Date of mailing of the international search report

22.11.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Tzschoppe, D

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/LV 96/00003

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 8940 Derwent Publications Ltd., London, GB; Class B05, AN 89-289767 XP002017003 & JP,A,01 213 259 (KYOWA HAKKO KOGYO KK) , 28 August 1989 see abstract ---	1-10
Y	ACTA BIOL. MED. GERM., vol. 35, no. 5, 1976, pages 645-656, XP002017002 E.STRACK ET AL.: "L-Karnitin als Basis cholinomimetischer Substanzen" see abstract -----	1-10

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/LV 96/00003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4382092	03-05-83	AU-A- 7908781	15-07-82
		BE-A- 891639	16-04-82
		CH-A- 649218	15-05-85
		DE-A- 3200016	12-08-82
		FR-A- 2497510	09-07-82
		GB-A, B 2091101	28-07-82
		JP-C- 1738989	26-02-93
		JP-B- 4024325	24-04-92
		JP-A- 57136516	23-08-82
		LU-A- 83869	07-05-82
		NL-A- 8200022	02-08-82
		SE-B- 453569	15-02-88
		SE-A- 8200007	07-07-82
-----			
US-A-5030458	09-07-91	AT-T- 105997	15-06-94
		AU-A- 7322791	26-06-91
		CA-A- 2045597	28-05-91
		DE-D- 69009176	30-06-94
		DE-T- 69009176	08-09-94
		EP-A- 0455808	13-11-91
		ES-T- 2054492	01-08-94
		JP-T- 4505400	24-09-92
		WO-A- 9107880	13-06-91
		US-A- 5186817	16-02-93
		-----	
US-A-4474812	02-10-84	BE-A- 898124	15-02-84
		CH-A- 655006	27-03-86
		DE-A- 3339052	03-05-84
		GB-A, B 2132085	04-07-84
		JP-A- 59098018	06-06-84
-----			
US-A-4451485	29-05-84	DE-A- 3234537	07-04-83
		FR-A- 2512671	18-03-83
		GB-A, B 2105992	07-04-83
		JP-C- 1363933	09-02-87
		JP-A- 58074606	06-05-83
		JP-B- 61029927	10-07-86
-----			

